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Dispersal patterns of meiospores shape population spatial structure of saxicolous lichens

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Abstract

Relationships between reproductive strategies and population spatial structure have been often suggested for lichens, but still not supported with experimental aerobiological data. For the first time, this study couples aerobiological investigations on meiospore dispersal by *Caloplaca crenulatella* (Nyl.) H. Olivier and *Rhizocarpon geographicum* (L.) DC. with the analysis of their local spatial patterns on the walls of a medieval castle in NW-Italy. During a two-year monitoring period carried out in the castle courtyard, a total of 169 polar diblastic spores, 20% of which were morphologically attributable to *C. crenulatella*, was detected in the mycoareosol, while muriform spores of *R. geographicum* were never found. Laboratory experiments confirmed that different dispersal patterns characterize the two species, the meiospores of *R. geographicum* being poorly discharged and only recovered at a short distance from the thalli, whereas those of *C. crenulatella* were more abundantly discharged, suspended and better dispersed by a moderate air flow. Such a difference was reflected on the castle walls by a random spatial pattern of *C. crenulatella*, while *R. geographicum* showed a clustered distribution. Different discharge rates and take-off limitations, possibly related to size differences between the spores, are not sufficient to explain the different colonization patterns and dynamics of the two species, and additional intrinsic and extrinsic factors likely drive the dispersal and establishment success. Nevertheless, information on the relationships between different dispersal patterns of the species and the local spatial structure of their populations may contribute to predict the recovery potential of lichen species exposed to habitat loss or disturbance, or encrusting monumental surfaces.

Key words: aerobiology, *Caloplaca crenulatella*, *Rhizocarpon geographicum*, spatial population patterns, stone cultural heritage, substrate preference

Introduction

Information on the structure and dynamics of lichen populations is fundamental to improve conservation effectiveness (Scheidegger & Werth 2009; Ellis 2012; Spitale & Nascimbene 2012), environmental monitoring techniques (Armstrong & Bradwell 2010; Giordani & Brunialti 2015), and Cultural Heritage management (Gazzano *et al.* 2009). Dispersal limitations and establishment constraints are addressed to explain the dynamics of lichen populations (Scheidegger & Werth 2009; Ellis 2012; Schei *et al.* 2012). Interactions between morphological and reproductive traits of different lichen species and micro-environmental conditions, including physical and chemical properties of substrates, microclimate and biotic features (Seaward 1977; Lawrey 1991; McIlroy de la Rosa *et al.* 2013; Nascimbene & Marini 2015), are suggested to shape spatial distribution patterns (Ellis & Coppins 2007; Spitale & Nascimbene 2012; Giordani *et al.* 2014). However, the dispersal and establishment patterns, and their significance for structuring species distribution, have only recently begun to be deeply explored, mainly focusing on epiphytic lichens (Scheidegger & Werth 2009; Ellis 2012; Leavitt & Lumbsch 2016).

Events of long-distance dispersal, favouring colonization of distant sites, followed by short-distance dispersal, supporting a more local population expansion, were shown to explain the patchy lichen colonization in a former tree-less landscape (Gjerde *et al.* 2015). The productions of large asexual diaspores (isidia, soredia) and small sexually-generated meiospores are traditionally related to local and long distance dispersal, respectively (Hedenås *et al.* 2003; Leavitt & Lumbsch 2016). At the landscape scale, inferences from genetic population studies and spatial pattern analyses mostly supported this view, suggesting a trade-off between a higher dispersal of meiospores, effective in landscapes with lower connectivity, and a higher establishment effectiveness of asexual diaspores in continuous landscapes (Ellis 2012). In-field measurements on diaspore dispersal generally showed short dispersal capabilities, but evidence on distributional ranges and phylogenetic studies suggested they are

also able to effectively disperse and successfully establish across broad distances (Leavitt & Lumbsch 2016). Accordingly, the propagule size was shown not to be a good predictor of population genetic structures in species of *Lobaria*, either at local or regional spatial scales (Werth *et al.* 2014).

In comparison to investigations on epiphytic lichens, experimental data on the colonization potential of saxicolous species are scarce, despite their importance in the debate for lichenometric applications (Loso & Doak 2006). Inferential analyses indicated an inconsistent relationship between the size of sexual meiospores and regional distribution of saxicolous lichens on gravestones (Leger & Forister 2009). However, Spitale & Nascimbene (2012) found a spatially structured pattern of lichen assemblages on calcareous boulders that could be partially influenced by dispersal effectiveness, even in the case of sexually-reproducing lichens. Moreover, spatial patterns at the local scale were mostly considered for mature saxicolous communities in terms of relationships between micro-habitat requirements and competition dynamics (John & Dale 1991; Jettestuen *et al.* 2010), but rather neglected with reference to dispersal and establishment dynamics.

Meiospore dispersal by saxicolous lichens has been mainly examined in controlled conditions in terms of rate, mode and distance of discharge (Pyatt 1973; Bailey 1976; Clayden 1997).

Recently, on the basis of an aerobiological approach, the dispersal of meiospores attributable to saxicolous lichens was quantified in the mycoaerosol of an alpine site (Favero-Longo *et al.* 2014). However, the relationship between the monitored meiospore dispersal and the local population structures was not analyzed.

This study aims at testing the hypothesis that the spatial structure of saxicolous lichen populations is affected by species limitations in propagule dispersal. Investigations were carried out in the courtyard of a medieval castle in NW Italy, where lichens are well established on both natural outcrops and the perimeter walls. We combined aerobiological monitoring data with the analysis of local distribution patterns of two selected species. In

particular, we examined, under field conditions, the dispersal patterns of two lichens with recognizable meiospores [*Rhizocarpon geographicum* (L.) DC. and *Caloplaca crenulatella* (Nyl.) H. Olivier], and we evaluated if these patterns are reflected by contrasting spatial population structures. Rates of meiospore discharge and dispersal, at short- and long-distance, were also examined in controlled laboratory conditions. How species-related establishment factors, for example in lithology preference, could combine with dispersal limitations in driving spatial colonization patterns was also considered. We expected that potential differences in the dispersal effectiveness of the two species should be reflected by different distribution patterns on the walls, lower and higher dispersal being reflected by aggregated and random distributions, respectively.

Material and Methods

Study site and lichens

Field investigations were carried out at the medieval castle of Graines (UTM ED50, N 5065923, E 403150; 1367 m a.s.l.; Brusson, Aosta Valley), in an alpine area of NW-Italy with a fairly dry climate regime (Verger 1992). The castle walls, about 250 m long and 10 m high, are located along the top of a steep relief, and surround ruined buildings, discontinuous stands of grassland and natural outcrops of base-rich siliceous rocks, mostly chloritoschists. The walls, built in four different phases beginning in the Middle Ages (XII, XV, XVI, XVII centuries) consist of irregularly shaped blocks of the chloritoschist bedrock and other local lithologies, bound with mortar (Sartorio 2012). A xerophytic lichen flora of 53 species was described for the site (Piervittori *et al.* 1991). The basiphilous species *Caloplaca crenulatella* (Nyl.) H. Olivier and the acidophilous *Rhizocarpon geographicum* (L.) DC., widely occurring on both the natural outcrops and the walls, and producing unequivocally recognizable small (12-16 x 6-7 μm) polar-diblastic and larger (22-40 x 10-19 μm) muriform spores, respectively, were considered for aerobiological and population analyses. Moreover, their

general distribution on the site was surveyed in plots (40 x 60 cm) randomly defined on the main rock outcrops (O1-O7) within the courtyard (n=70 plots, 10 plots per outcrop) and on differently oriented wall transects (S1-S10) facing the courtyard (n=56, 5-7 plots per transect) (Fig. 1). For each plot, frequency and abundance, computed as percentage cover, of the two species, and of other species of the genus *Caloplaca*, were visually estimated. Remote observations of the inaccessible external side of the walls were also randomly conducted from embrasures (E1) and a temporary scaffolding (E2).

Aerobiological monitoring

The airborne concentration of meiospores of *R. geographicum* and *C. crenulatella* was sampled at the castle of Graines during a two-year monitoring program between June 2011 and June 2013. Air samples were continuously collected (10 L min^{-1}) one week per month using a 7-day recording Hirst-type volumetric sampler VPPS 2010, suitable for particles in a dimensional range of 5 to $100 \mu\text{m}$ (Lanzoni, Bologna, Italy), located at 1.5 m above the ground level in the castle courtyard (Fig. 1), where the speed and direction of wind were monitored using a Sonic Young 81000 anemometer (RM Young, Traverse City, MI, USA). The adhesive-tape on which airborne particles had been impacted was examined according to UNI 11108:2004 (see Travaglini *et al.* 2009) and the spore concentration calculated 6 days per month. Polar diblastic spores of *C. crenulatella* were distinguished from those of other local Teloschistaceae on a morphological basis (length x width, septum thickness; see Favero-Longo *et al.* 2014 for additional details).

The spore dispersal pathways of the two lichen species were also examined in controlled conditions using a microcosm device to estimate discharge, short- and long-range dispersal of spores (Favero-Longo *et al.* 2014). To estimate spore discharge, ten rocks colonized by *R. geographicum* (av. total cover per rock: 5.4 cm^2 , of thalli with diameters of 5-15 mm; av. apothecia per cm^2 : $24 \pm 10 \text{ SD}$) and five colonized by *C. crenulatella* (av. total cover per rock:

0.2 cm², of thalli with diameters of 2-4 mm; av. apothecia per cm²: 47±5 SD) were immersed for 15 min in deionized water and incubated for six hours, covered with Petri dishes containing agar medium (Petri diameter = 5.5 cm; agar surface 6 mm above the thalli), within a perspex box (120 x 70 x 60 cm³) including a fan moving the air at about 4 m sec⁻¹ on the rock surfaces. To estimate spore dispersal, four groups of rocks colonized by *R.*

geographicum (total cover on the first and second rock groups: 60 and 200 cm², of thalli with diameters of 5-15 mm) and *C. crenulatella* (total cover on the third and fourth rock groups: 40 cm² each, of thalli with diameters of 2-18 mm), respectively, were immersed for 15 min in deionized water and incubated for three days within the perspex box including the fan moving the air at about 4 m sec⁻¹ on the rock surfaces, 10 Petri dishes (diameter = 5.5 cm) containing agar medium and the VPPS sampler. The Petri dishes, encircling the rocks at a distance of 5-15 cm from the colonized surfaces, were used as passive traps to estimate short-range dispersed meiospores. With the VPPS, located with the suction nozzle at 40 cm from the ground layer and at 50-70 cm from the colonized surfaces, the meiospore dispersion in the air volume was examined, the spore take-off and entering the turbulent air being considered a requisite for long-range dispersal. The meiospores impacted on the agar surfaces and on the VPPS adhesive tape were quantified at 400x magnification according to Favero-Longo *et al.* (2014).

Population structure survey and analysis

The spatial structure of *C. crenulatella* and *R. geographicum* populations on the walls was surveyed along four vertical transects (w x h: 2.5 x 10 m), distributed at 15-25 m from the aerobiological monitoring point on the north-facing inner side of the castle, notably covered by lichens, accessible using a temporary scaffolding and sharing similar microclimatic conditions. The occurrence and abundance of the two species were assessed on each of the 1593 blocks of the wall transects, classified on the basis of the lithology as follows: base-rich

siliceous (chloritose schists, with subordinate serpentinites and metabasites: 86%) >> acid siliceous (amphibolite gneisses: 13%) >> basic calcareous (calcschists: 1%) blocks, with each rock type displaying a homogeneous spatial distribution. The specific abundance was estimated according to ordinal cover scales (*R. geographicum*: 4 = >50%, 3 = 26-50%, 2 = 3-25%, 1 = <3%, 0 = no cover; *C. crenulatella*: 4 = >30%, 3 = 11-30%, 2 = 1-10%, 1 = <1%, 0 = no cover). The diameter of the largest *Rhizocarpon* thallus was measured on 108 randomly selected blocks with cover values higher than 3%, distributed among the four different building phases according to available annotations on the wall perspective drawings. Such measures of the thalline size were not performed for *C. crenulatella*, because of frequent difficulties in reliably recognizing large individual thalli from coalescent ones, particularly in the case of more covered surfaces. The presence of apothecia and mature spores inside the asci was verified for the following diameter classes of *R. geographicum* thalli: <3 mm (n=12), 4-6 mm (n=8), 10-12 mm (n=6), 13-17 mm (n=5), and for 12 thalli of *C. crenulatella* without distinguishing dimensional classes. All the spatial information, together with the classification of blocks with reference to their lithotype and their colonization by the two species, was mapped and analyzed using GIS 9.3 (ESRI, Redlands, CA, USA).

We tested the hypothesis of random lichen distribution with respect to the rock substrate by using the univariate and bivariate pair correlation function (g), a second order statistic closely related to Ripley's (K) function. The g function is non-cumulative respect to the K one, this means that it uses only points separated by a certain distance r allowing, in this way, specific scales to be identified where significant point-point interactions occur (Wiegand *et al.* 2007). The selection of the correct null model represents a key step for the unbiased interpretation of the results. Here we applied the random labelling null model or the antecedent condition in case of bivariate analysis in order to consider the rock type distribution fixed before the arrival of the lichens (Aakala *et al.* 2012; Wiegand & Moloney 2013). In practice, we contrasted the presence/absence data of the two species with the corresponding rock type of

the wall. In this analysis, we considered only the two most common rock types (base-rich siliceous and acid siliceous).

For all the analyses, significant departure from the null models was evaluated based on 95% simulation envelopes, which were calculated from the 5th-lowest and 5th-highest values of 99 Monte Carlo simulations. A distribution is classified as clumped, random or regular for univariate analysis, when the value is located above, inside or below the 95% confidence intervals, respectively. Similarly, for bivariate analysis, two populations are significantly positively correlated (attraction), spatially independent or significantly negatively correlated (repulsion), when the value is located above, inside or under the 95% confidence intervals, respectively.

All univariate and bivariate point pattern analyses were performed using the grid-based software Programita and applying a distance class of 0.5 m (Wiegand & Moloney 2004, 2013). We conducted the spatial analyses considering the 4 plots as replications since we were interested in the average spatial pattern (Raventós *et al.* 2010). Results of the four plots have been thus combined in one average graphic function, using the 'combine replicates tool' included in the Programita software (Wiegand & Moloney 2004, 2013).

The global coefficient Moran's I (Moran 1950), a product-moment like coefficient computing autocorrelation between variables as a function of distance lags, was used to evaluate whether or not the two species are distributed according to a clustered spatial structure. The global significance of the correlogram was evaluated at the significance level $\alpha' = \alpha/v$ according to the Bonferonni criterion to correct for multiple tests. Correlograms were arranged plotting the values of Moran's I in the y-axis against the distance among points in the x-axis.

The spatial structure of the lichen populations was further analyzed with Local *Gi statistics that calculates the similarity of each point to its neighbors in a specified radius d including the target point into the computation. The graphical representation of Local *Gi is able to locate

hot spots and gaps in lichen distribution (Getis and Ord 1992, 1996). Global and local autocorrelation were computed with the Excel Add-Inn RookCase (Sawada 1999).

Results

Lichen distribution at the study site

Outcrops within the courtyard displayed an average lichen cover around 80%, dominated by xerophytic silicicolous crustose species [e.g. *Aspicilia caesiocinerea* (Malbr.) Arnold, *Rhizocarpon geographicum* (L.) DC., *Lecidella carpathica* Körb., *Candelariella vitellina* (Hoffm.) Müll. Arg.]. *R. geographicum* widely occurred, showing relatively high cover values (4-8%) on undisturbed outcrops (O2, O3, O4, O6), with low cover (<1%) on frequently trampled surfaces (O1) and where mosses and plants were abundant (O5, O7) (Fig. 2A; Fig. S1). *Caloplaca crenulatella* and other three species of the genus *Caloplaca* [*Caloplaca crenularia* (With.) J.R. Laundon, *C. saxicola* gr. and *C. rubroaurantiaca* de Lesd.] also characterized the lichen vegetation, showing higher frequency and abundance on the trampled plot (O1).

Lichens also colonized parts of the buildings and the walls facing the courtyard (Fig. 2B). Colonization was relatively abundant on the north-facing walls (S1-S2), where *R. geographicum* displayed its highest cover. *C. saxicola* and *C. crenulatella* dominated building walls where soil and plants abundantly filled the cracks between the rock blocks (S5-S6). By contrast, lichen colonization was quite absent on southern- (S7-S8) and eastern- (S9-S10) facing walls and on the recently restored chapel walls (S3-S4). Qualitative observations on the external side of the walls also showed high lichen cover and *R. geographicum* occurrence on the north-facing surfaces (E11), while lower lichen abundance was found on the south-facing surfaces (E12).

Meiospore dispersal

A total of 169 polar-diblastic spores, unequivocally attributable to Teloschistaceae, were found in the mycoaerosol sampled at the castle of Graines (Table 1A). Their detection was not evenly distributed during the two-years monitoring period, but concentrated in 12.5% of the monitored days, distributed in 9 months. A positive relationship between the presence of polar-diblastic spores in the air spora and the occurrence of rainfall events in the previous 24h was found (Table S1). The wind, mostly blowing from the north-west quadrant, displayed an average speed of 2.1 m sec^{-1} during the two-years monitoring, but relatively recurrent events of wind gusts (at least one event of wind gusts $>40 \text{ m sec}^{-1}$ in 6 out of the 9 months during which the polar-diblastic spores were observed).

The daily concentrations of polar-diblastic spores ranged between 0.3 and $16.3 \text{ spores m}^{-3}$ (Table 1B), but in all the cases they represented a minimal part of the total airborne spores (av. $1372 \text{ spores m}^{-3}$, as calculated during the first monitoring year). On the basis of their morphology, 20% of the detected polar-diblastic spores were attributable to *C. crenulatella*, captured in 7.6% of the monitored days, while the others could be assigned to the other Teloschistaceae occurring in the castle courtyard: *C. crenularia* (45%), *C. saxicola* gr. or *Xanthoria elegans* (Link.) Th. Fr. (25%) and *C. rubroaurantiaca* (9%) (Table 1C).

Muriform spores attributable to *Rhizocarpon* on the basis of morphology were never detected in the mycoaerosol during the whole monitoring period.

Accordingly, spore dispersal experiments in controlled conditions showed different patterns for thalli of *C. crenulatella* and *R. geographicum* with apothecia bearing mature spores (Fig. 3). In the monitored microcosm, the polar-diblastic spores of *C. crenulatella* were abundantly discharged (2411 ± 243 spores released per cm^{-2} of thallus, equal to approx. 50 spores per incubated apothecium) (Fig. 3A). They were found on the agar surfaces at a short distance from the colonized rocks (av. 2.7 spores per cm^{-2} of agar exposed at 5-15 cm, equal to an av. total of 621 spores collected on 10 agar plates; Fig. 3B) in an amount which is estimated equal to 0.6% of the total spores discharged from the incubated *Caloplaca* thalli on the basis of the

discharge analysis (*i.e.* approx. 96000 spores from 40 cm² of thalli). The *Caloplaca* spores were also detected in the air collected by the volumetric sampler (av. 72 spores m⁻³, equal to an av. total of 3110 spores collected during the whole monitoring; Fig. 3C), in an amount which is estimated equal to 3.2% of the total spores discharged from the incubated *Caloplaca* thalli on the basis of the discharge analysis. Most of the spores observed on the agar surfaces were still organized in packages of 5-8 spores, while those collected by the Hirst-type sampler singularly impacted the adhesive-tape. By contrast, the large muriform spores of *R. geographicum* were poorly discharged (av. 1.8 spores released per cm⁻² of thallus, equal to approx. 0.1 spores per incubated apothecium; Fig. 3A). Accordingly, they were sporadically detected on the agar surfaces exposed around the incubated thalli (av. 0.03 spores cm⁻² of agar exposed at 5-15 cm, equal to an av. total of 6 spores collected on 10 agar plates; Fig. 3B), in an amount which is estimated equal to 2.6% of the total spores discharged from the incubated *Rhizocarpon* thalli on the basis of the discharge analysis (approx. 230 spores from av. 130 cm² of thalli). Moreover, *Rhizocarpon* spores were never collected by the volumetric sampler (Fig. 3C).

Population spatial structure

The frequency of the blocks of the north-facing castle wall colonized by *C. crenulatella* and *R. geographicum* was comparable (approx. 80%) (Table 2). *R. geographicum* frequently had cover values higher than 25% (14% of blocks), with thalli distributed through the whole surface of blocks. By contrast, *C. crenulatella* mostly colonized the block margins in proximity of mortar (and the mortar itself), rarely covering more than 10% (2% of blocks). *Rhizocarpon* thalli had rather small homogeneous size, even on the more colonized blocks: a mean diameter of 7.9 ± 3.2 mm of the largest thalli was indeed recorded without significant differences between the blocks of the wall sectors attributable to the different building phases. Largest diameters between 15 mm and 17 mm were observed (5.5% of the surveyed pool of

largest thalli). Apothecia were observed also in thalli with diameter <3 mm, but fertile spores were only observed in higher diameter classes, the highest abundance of spores being observed in largest thalli. Thalli of *C. crenulatella*, from extremely small (diameter of approx. 1 mm) to relatively large (diameter >10 – 15 mm), were abundantly covered by fertile apothecia.

The frequency of *R. geographicum* was similar on the acid-siliceous and base-rich siliceous blocks (77 and 79%, respectively), but higher cover values mostly characterized acid-siliceous blocks (cover values $>25\%$ on 24% of acid-siliceous blocks vs. 13% of base-rich siliceous ones). *Caloplaca* frequency and abundance were instead relatively higher on the base-rich siliceous blocks. However, the random distribution depicted by the pair correlation function indicated that the presence of the two species along the wall transects was not significantly influenced by the block lithologies (Fig. 4).

On the other hand, analysis of spatial community structure, computed as Moran's indices, showed contrasting distribution patterns between the two species (Fig. 5). *R. geographicum* displayed Moran's index significant values ranging between $+15$ and -20 , indicating for the thalli a trend to form clusters (scale 1 m) separated by uncolonized areas (approx. 2m). By contrast, Moran's index values calculated for *C. crenulatella* were almost below the threshold of significance, indicating a randomly dispersed rather than aggregated distribution of thalli. Local *G statistics also showed for *R. geographicum* that blocks having cover values higher than the average were mostly aggregated (Fig. 6A), while in the case of *C. crenulatella* a more dispersed, random pattern of distribution was observed (Fig. 6B). Moreover, the dense colonies of *Rhizocarpon* were mostly concentrated in the lower part of the wall.

In some cases, few distinct large thalli (diameter > 1 cm) of *R. geographicum* were surrounded on the same block or on surrounding blocks by several thalli of lower diameter classes (Fig. 7A). In several other cases, rocks or rock groups with high cover of *R. geographicum*, but poorly distinguishable individuals, were located above other rocks with

lower covers and well distinguishable distinct thalli of lower diameter classes (Fig. 7B).

Similar patterns of aggregation were not observed for *C. crenulatella*.

Discussion

To our knowledge, this is the first study that combined analyses on the population spatial structure of saxicolous lichens with experimental evidences on meiospore dispersal patterns obtained with an aerobiological approach. Results confirm our main expectations, indicating that differences in the specific dispersal patterns are reflected by different distribution patterns. The occasional detection of the meiospores of *Caloplaca crenulatella* in the myco-aerosol is reflected by a random distribution pattern of the species. On the contrary, *Rhizocarpon geographicum*, which exhibited a clustered distribution pattern, is likely affected by dispersal limitation, as suggested by the fact that its meiospores were never detected in the myco-aerosol. However, this difference does not reflect the relative abundance of the two species in the study site, as *R. geographicum* displayed equal frequencies and higher cover values than *C. crenulatella* on the blocks of the wall, and was also more abundant on the natural rock outcrops (Favero-Longo *et al.* 2014). This fact suggests that, on the examined wall, the richer pool of dispersed meiospores of *C. crenulatella* may experience higher establishment limitations than that of *R. geographicum*. Intrinsic and extrinsic factors possibly related to the dispersal and establishment limitations of *R. geographicum* and *C. crenulatella*, and potentially influencing their population structures, are discussed in the following sections. It is worth noting here that the relatively small size of the largest *Rhizocarpon* thalli, compatible with an average age in the order of decades (Armstrong & Bradwell 2010), indicated an early stage of lichen colonization. This suggests a “modern age” recolonization phase after the restorations ended in the 1910s and 1960s (Sergi 2012). Accordingly, the parts of the walls attributable to the different building phases of the XII-XVII centuries host *Rhizocarpon* thalli of comparable size.

Dispersal limitations

The dispersal of a sufficiently large propagule pool is the precondition to found and expand new lichen populations (Scheidegger & Werth 2009). Although a main research focus has been paid to dispersal distances and their influence on species distribution and population structures (Johansson *et al.* 2012; Werth *et al.* 2014; Gjerde *et al.* 2015; Leavitt & Lumbsch 2016), successful dispersal definitely depends on the whole aerobiology pathway, including propagule discharge, take-off, transport (dispersal *s.s.*), deposition and impact (Lacey 1996; Magyar *et al.* 2016).

In this work, we observe for *R. geographicum* a three orders of magnitude lower discharge rate (as spores released per cm² of thallus) than in *C. crenulatella*, which clearly poses higher initial limitations for the dispersal success of the species. Ascocarp occurrence for the two species was relatively similar (two-times higher in *Caloplaca*) and fertile ascocarps were abundantly observed also in very young *Rhizocarpon* thalli (diameters of 4-6 mm). However, microscopy observations generally showed a strongly higher spore production in the *Caloplaca* ascocarps (data not shown) and ejection defects have been already reported in *R. geographicum* (Bailey & Garrett 1968; Crittenden *et al.* 1995), which moreover displays different ascus anatomy and dehiscence type (Bellemère & Letrouit-Galinou 1988), likely explaining the recorded differences in the discharge rate.

In addition, *R. geographicum* displays meiospores larger than those of *C. crenulatella*. Although the relatively large size of *Rhizocarpon* spores is not comparable with that of vegetative diaspores and certain huge meiospores (as those of *Pertusaria*), these size divergences were suggested to influence dispersal patterns and to explain specific metapopulation dynamics in lichen epiphytes (Johansson *et al.* 2012).

In the absence of air movement in previous studies, specific differences in the distance and rate of lichen meiospore horizontal discharge did not show a clear relationship with spore size

(Bailey & Garrett 1968), although packages of more spores are generally better dispersed than single spores (Garrett 1971). However, spore discharge in the environment exposes spores to air turbulence (wind), which influences the behaviour of particles in the atmosphere (De Nuntiis *et al.* 2003). In our laboratory experiment, spore packages of *C. crenulatella* and relatively large spores of *R. geographicum* were discharged, but not suspended by the air, thus settling at a short distance from the discharge source. In contrast, the collection of the single spores of *C. crenulatella* by the volumetric sampler indicates their take-off and dispersion through the whole volume of the experimental device. If the discharged spores of *Rhizocarpon* would have been suspended and captured with the same rate of those of *Caloplaca* (3.2% of putatively discharged spores captured by the volumetric sampler), we should have also found a detectable amount of *Rhizocarpon* spores (approx. 5-10). However, no *Rhizocarpon* spores were collected by the volumetric sampler either in the laboratory experiment or in the field monitoring, where the polar-diblastic spores were instead periodically found (8% of monitored days, related to rainfall events). This suggests that further factors may contribute to limit the dispersal of *R. geographicum* other than the poor discharge. Depending on different dimensions and consequent settling velocities, a four times lower lifetime in the atmosphere is expected for single spores in the dimensional range of *R. geographicum*, or for the large packages of *C. crenulatella* spores, compared to those of *C. crenulatella* (De Nuntiis *et al.* 2003). However, models of spore trajectories suggest that values of settling velocity expected for the dimensional ranges of *C. crenulatella* (in the order of 0.004-0.008 m sec⁻¹ for diameters of 12-16 µm; De Nuntiis *et al.* 2003) and *R. geographicum* (in the order of 0.03-0.05 m sec⁻¹ for diameters of 30-40 µm; De Nuntiis *et al.* 2003) should not significantly influence the dispersal distance once the spores are suspended in the air (Kuparinen *et al.* 2007). On the other hand, spore dimension may be also related to the take-off velocity, as different lift forces from the flow may be required to exceed the particle weight and adhesive forces holding the particle down (Descamps *et al.* 2005).

However, take-off velocity has been still poorly investigated in aerobiology and, in particular, about fungal spores (Noblin *et al.* 2009), so that we can only speculatively hypothesize its influence on the *R. geographicum* dispersal limitation.

Whatever the limitation factors and their combined effects, *C. crenulatella* displays the potential for both a short-range dispersal of spore-packages and an occasional long-range dispersal of the small single spores, while *R. geographicum* potential dispersal seems rather limited at a short range. *C. crenulatella* thus exhibits a behaviour similar to that of some epiphytic lichens, for which events of long- and short-range dispersal were shown to contribute in parallel to the colonization of distant sites and to more local population expansions, respectively (Gjerde *et al.* 2015). Such efficient dispersal pattern is consistent with the homogeneous distribution of the species throughout the examined north-facing wall. By contrast, the patchy distribution of *R. geographicum* and observations of its progeny surrounding largest thalli or more colonized blocks suggests that scattered founder thalli established on the wall upon rare/exceptional events of effective long-distance dispersal, promoting colonization in their proximity (short-distance dispersal) after reaching reproductive maturity. At the patch level, run-off water may also significantly contribute to the meiospore short-distance dispersal (Armstrong 1981).

In this framework, dispersal-limits of vegetative diaspores of some epiphytic lichens, with dimensions up to one order of magnitude larger than those of *R. geographicum*, have been estimated in the order of tenths of meters on the basis of indirect evidences (Ellis 2012), but the frequency of dispersal events is unknown. Notably, the only aerobiological assessment of abundant soredia dispersal is available for the saxicolous communities of Maritime Antarctic (Marshall 1996), exposed to extreme windy conditions if compared with those of the study site. However, a comparison between vegetative diaspore and meiospore dispersal should take into account the opposite conditions favouring the propagule release, as meiospore dispersal

follows rainfall events (Favero-Longo *et al.* 2014), while vegetative diaspore release is related to low relative humidity (Marshall 1996).

Establishment limitations

Meiospores of other *Caloplaca* species colonizing the outcrops in the courtyard (Favero-Longo *et al.* 2014) were found in the myco-aerosol with comparable amounts as those of *C. crenulatella*. However, only *C. saxicola* was sporadically found on the wall transects, while *C. crenularia* occurred in differently exposed sides of the walls and *C. rubroaurantiaca* was not found at all (Piervittori *et al.* 1991; Favero-Longo *et al.* 2014). Factors others than dispersal limitation should thus contribute to select the species which colonize the examined parts of wall and determine their abundance, likely affecting the establishment step (*i.e.* the effective dispersal; Scheidegger & Werth, 2009).

The preference for lithology (Brodo 1973; Spitale & Nascimbene 2012) should not exclude the other *Caloplaca* species from the wall, as they colonize the chloritoschist on the outcrops. Moreover, the random labelling null model indicates that the block lithologies do not significantly affect the presence-absence distribution of the the acidophytic *R. geographicum* and the basiphytic *C. crenulatella* on the wall blocks. However, the block-scale distribution of *C. crenulatella* at the block margins agrees with a positive influence of the mortar proximity on its establishment success. Its higher establishment limitation far from the block-margins likely accounts for the general poor cover values, apparently contrasting the high rates of spore dispersal. By contrast, high covers by *Rhizocarpon* in the surrounding of founder individuals develop on the whole surfaces of the siliceous rock blocks, mostly on acid-siliceous ones, suggesting a high establishment success of the low number of larger dispersed propagules. This may agree with the fact that reproductive structures with higher mass generally show higher survival probability, but a similar pattern still needs to be verified for lichen meiospores (Werth *et al.* 2014).

Higher lichen colonization on the examined north-facing side of the walls agrees with the dominant wind direction from the north-west quadrant, possibly supporting spore transport from the outcrops within the courtyard. However, several other factors, as light, moisture and nitrogen availability, not considered in our study, may also drive the observed population patterns by affecting the establishment step (Prieto *et al.* 1999), as suggested by higher *Caloplaca* covers on the east- and south-facing walls with high plant covers.

A major localization of the *R. geographicum* populations in the lower parts of the wall transects may be related to the height of dispersal trajectories of spores rarely dispersed from the rock outcrops (Kuparinen *et al.* 2007), but the potential dispersal by animals living at the ground level should be also considered, although this phenomenon has still received poor experimental support (Lawrey 1984; Scheidegger & Werth 2009; Boch *et al.* 2011).

Moreover, higher humidity in the lower part of the wall may favour the few dispersed large meiospores to meet a higher amount of free-living photobionts, which are another limiting factor for successful lichen establishment (Scheidegger & Werth 2009; Sanders 2014; Belinchón *et al.* 2015). Higher humidity at the bottom of the wall may enhance the motility of algal zoospores involved in the formation of primary areolae of *Rhizocarpon* thalli (Armstrong & Bradwell 2010).

Concluding remarks

Our results revealed that contrasting patterns of spore dispersal influence lichen spatial distribution during the early stages of colonization. Species poorly dispersed because of limitations in meiospore discharge, take off and/or transport are expected to have an aggregated distribution, while species more effectively dispersed are expected to have a random distribution pattern. Although other extrinsic and intrinsic factors may pose establishment limitations and affect colonization dynamics and patterns, this result should be considered with regard to the consequences of habitat disturbance/loss or when strategies to

support (Smith 2014) or contrast (Seaward 2015) the lichen occurrence on natural or cultural-heritage are planned. Species behaving as *C. crenulatella* are likely to rapidly and diffusely (re-)colonize suitable substrates if fertile thalli persist in the nearby areas or are reintroduced. Species with these traits should better tolerate local and/or temporary disturbance (e.g. forest management) and their transplantation could represent a suitable approach to restore their populations in previously impacted habitats. On the other hand, their removal from monuments is expected to be poorly effective in the long term, if populations still occur in the nearby areas. On the contrary, species behaving like *R. geographicum*, although displaying a relatively efficient local population expansion, need longer periods to spread far from already established thalli. Accordingly, the temporary and/or local disturbance of their habitat and its fragmentation may strongly impact their distribution and their transplanted thalli are expected to be less effectively used in restoration practices. On the other hand, these species are expected to be easily controlled on monuments, even if populations still occur in the surroundings.

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Table 1. Polar diblastic spores in the mycoaerosol of the castle of Graines (June 2011-June 2013). A, total counts of polar diblastic spores. B, daily concentration (spores m^{-3}). C, percentage of spores attributable to the different Teloschistaceae species occurring in the site on the basis of morphological features (length x width, septum length). D, wind conditions during the monitored month (*, above the instrumental limit). No spores were observed in the months which are not indicated in the table.

Polar-diblastic spores		2011				2012			2013		Total
		Jul	Aug	Oct	Feb	Aug	Sep	Mar	Apr	May	
A	Total counts	9	9	9	8	58	31	3	38	4	169
B	Spores m^{-3} - Day1	-	1.0	-	-	1.7	-	-	-	-	
	Day2	3.1	-	3.1	-	1.4	2.8	-	-	1.4	
	Day3	-	-	-	-	-	-	-	5.2	-	
	Day4	-	-	-	-	16.3	-	-	3.8	-	
	Day5	-	-	-	-	0.7	8.0	1.0	2.1	-	
	Day6	-	1.7	-	2.8	-	-	-	2.1	-	
C	<i>Caloplaca crenulatella</i> (%)	33.3	44.4	0.0	0.0	19.0	25.8	0.0	21.1	0.0	20.1
	<i>Caloplaca saxicola</i> (%)	33.3	11.1	0.0	75.0	24.1	16.1	100.0	18.4	100.0	25.4
	<i>Caloplaca crenularia</i> (%)	0.0	0.0	11.1	25.0	55.2	58.1	0.0	60.5	0.0	45.0
	<i>Caloplaca rubroaurantiaca</i> (%)	33.3	44.4	88.9	0.0	1.7	0.0	0.0	0.0	0.0	9.5
D	Av. wind speed (m sec^{-1})	1.1	1.0	1.8	3.5	1.2	1.1	3.7	1.6	1.3	
	Max. wind speed (m sec^{-1})	7.4	5.1	>40.0*	>40.0*	7.0	>40.0*	>40.0*	>40.0*	>40.0*	
	Av. wind direction (°)	18.1	6.8	-6.6	-12.0	25.5	1.1	15.5	29.9	21.1	

Table 2. Frequency and abundance of *Rhizocarpon geographicum* (A) and *Caloplaca crenulatella* (B) on the blocks of the castle wall. The % frequency of different cover values on the different block lithologies is shown with reference to the ordinal scales (CI_0/CI_4) indicated for each species.

		blocks (n)		Frequency (%)				
		CI_0	CI_1	CI_2	CI_3	CI_4	Total	
A	<i>Rhizocarpon geographicum</i> cover values	0%	<3%	3-25%	26-50%	>50%		
	Overall blocks	1593	22.4	16.9	46.0	13.4	1.3	77.6
	Acid siliceous blocks	205	23.4	15.6	36.6	20.0	4.4	76.6
	Base-rich siliceous blocks	1372	21.4	17.3	47.9	12.6	0.8	78.6
	Basic-calcareous blocks	16	93.8	0.0	6.3	0.0	0.0	6.3
B	<i>Caloplaca crenulatella</i> cover values	0%	<1%	1-10%	11-30%	>30%		
	Overall blocks	1593	20.7	47.8	29.5	2.0	0.0	79.3
	Acid siliceous blocks	205	34.6	51.2	12.7	1.5	0.0	65.4
	Base-rich siliceous blocks	1372	18.6	47.6	31.8	2.0	0.0	81.4
	Basic-calcareous blocks	16	18.8	25.0	50.0	6.3	0.0	81.3

Figure captions

Fig. 1. Topography of the Castle of Graines and location of main rock outcrops within the walls (O1-O7), wall transects (S1-S10 and T1-T4) and external areas (E1-E2) surveyed for lichen colonization. Hirst volumetric sampler (HS); meteorological station (MS), including the sonic anemometer. Scale bar: 5 m.

Fig. 2. Distribution of *Rhizocarpon geographicum*, *Caloplaca crenulatella* and other *Caloplaca* species in the study site. A, specific cover and frequencies on the rock outcrops within the courtyard. B, specific cover and frequencies on wall transects with different cardinal-facing directions. Location of natural plots and wall transects is mapped in Fig. 1.

Fig. 3. Spore dispersal by *Caloplaca crenulatella* and *Rhizocarpon geographicum* in the laboratory tests. A, spore discharge. B, spores collected on agar surfaces exposed at 5-15 cm from rock blocks colonized by the two species (short-range dispersal). B, spores suspended by the air-flow and collected by the Hirst-type volumetric sampler (long-range dispersal).

Fig. 4. Example of the graphical results of the bivariate spatial point pattern analysis with the pair correlation function (g). The presence of the species along the wall transects on the medieval castle of Graines was clearly not significantly influenced by the block lithology considering that the resulting function (thick black line) always falls within the 95% confidence envelope defining the random distribution area. Since the plots obtained for the two species on the four vertical transects on the wall are similar we report here only one of the eight plots.

Fig. 5. Moran's I correlogram for the two species surveyed on the stones along the four vertical transects on the wall of the medieval castle of Graines. Dashed black lines indicate significance levels ($p < 0.05$). Dark grey lines: *Rhizocarpon geographicum*; light grey lines: *Caloplaca crenulatella*.

Fig. 6. Local $*Gi$ bubble plots for *Rhizocarpon geographicum* (A) and *Caloplaca crenulatella* (B) on the stones along the four vertical transects on the wall of the medieval castle of Graines (axis ticks = 1 m). Bubble size is proportional to the intensity of autocorrelation at a given location. Dark grey bubbles indicate stones where the abundance of the species is higher than the mean values, while light grey bubbles indicate stones where the abundance of the species is lower than the mean values. White bubbles indicate stones where the abundance of the species is not significantly different from the mean values.

Fig. 7. Small-scale spatial distribution of *Rhizocarpon geographicum*. A, thalli with larger diameters (approx. 10 mm, ***) are surrounded by a progeny of thalli of lower dimensions (diameters approx. 6 mm, **, and <2 mm, *). B, C, rock blocks with high *Rhizocarpon* cover above blocks with decreasing cover and thallus dimensions at increasing distances (arrows).

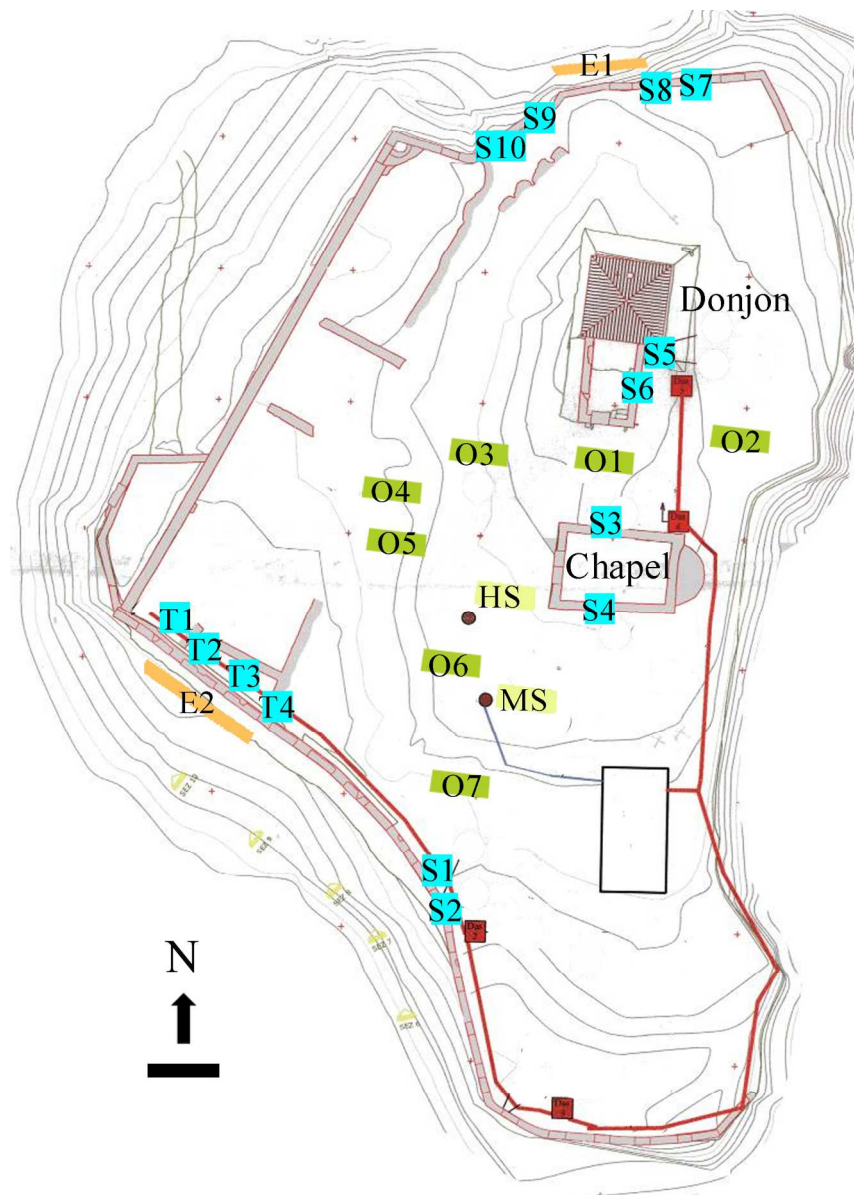


Fig. 1. Topography of the Castle of Graines and location of main rock outcrops within the walls (O1-O7), wall transects (S1-S10 and T1-T4) and external areas (E1-E2) surveyed for lichen colonization. Hirst volumetric sampler (HS); meteorological station (MS), including the sonic anemometer. Scale bar: 5 m.

Fig. 1

91x126mm (600 x 600 DPI)

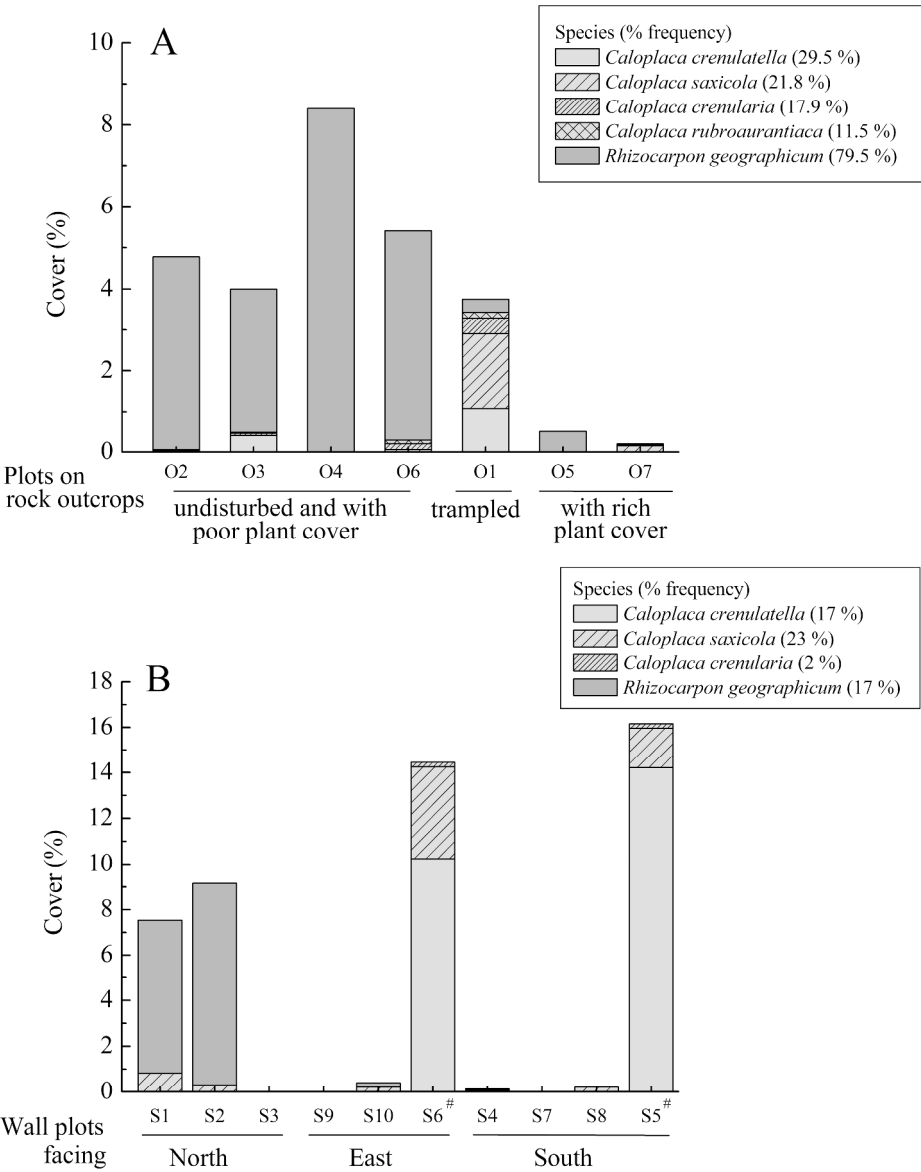


Fig. 2. Distribution of *Rhizocarpon geographicum*, *Caloplaca crenulatella* and other *Caloplaca* species in the study site. A, specific cover and frequencies on the rock outcrops within the courtyard. B, specific cover and frequencies on wall transects with different cardinal-facing directions. Location of natural plots and wall transects is mapped in Fig. 1.

Fig. 2

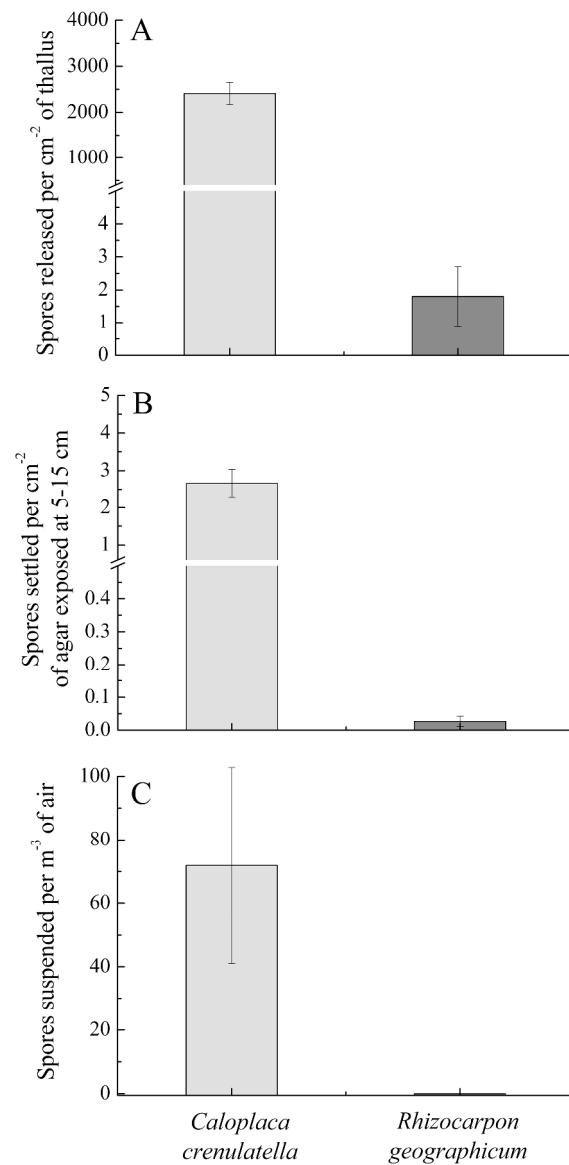


Fig. 3. Spore dispersal by *Caloplaca crenulatella* and *Rhizocarpon geographicum* in the laboratory tests. A, spore discharge. B, spores collected on agar surfaces exposed at 5-15 cm from rock blocks colonized by the two species (short-range dispersal). C, spores suspended by the air-flow and collected by the Hirst-type volumetric sampler (long-range dispersal).

Fig. 3

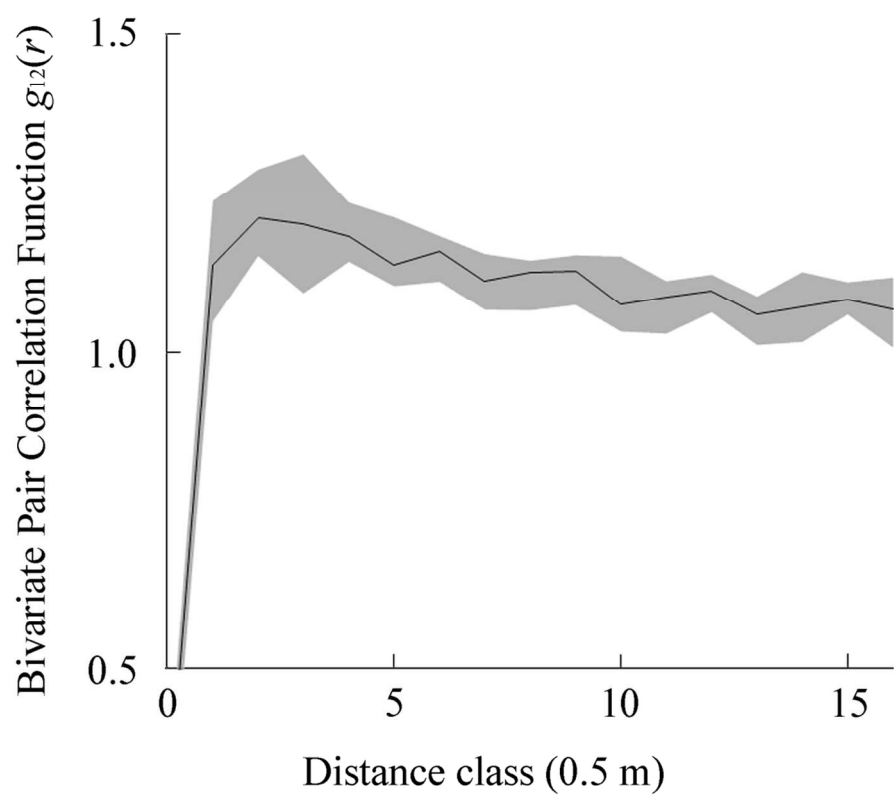


Fig. 4. Example of the graphical results of the bivariate spatial point pattern analysis with the pair correlation function (g). The presence of the species along the wall transects on the medieval castle of Graines was clearly not significantly influenced by the block lithology considering that the resulting function (thick black line) always falls within the 95% confidence envelope defining the random distribution area. Since the plots obtained for the two species on the four vertical transects on the wall are similar we report here only one of the eight plots.

Fig. 4
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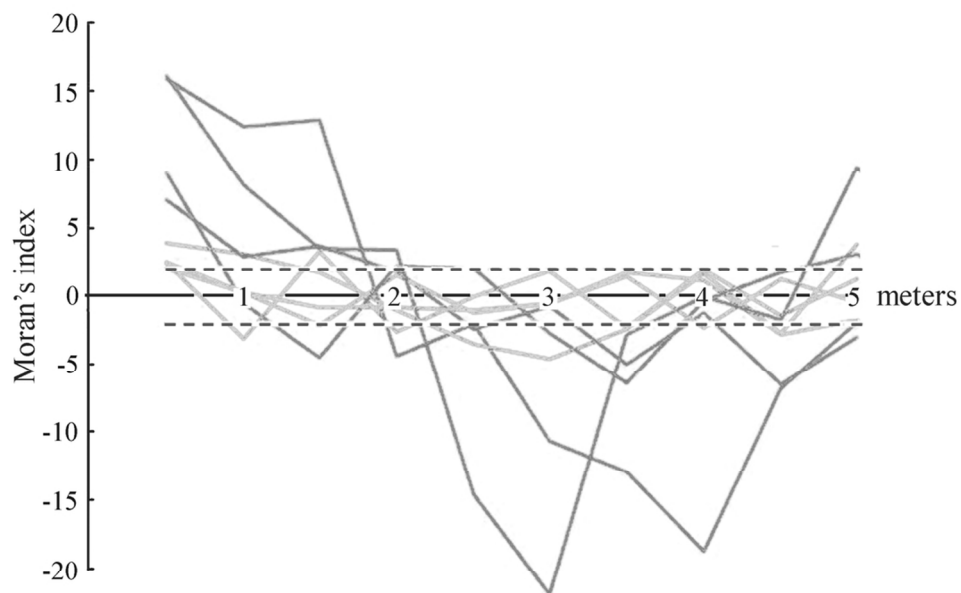


Fig. 5. Moran's I correlogram for the two species surveyed on the stones along the four vertical transects on the wall of the medieval castle of Graines. Dashed black lines indicate significance levels ($p < 0.05$). Dark grey lines: *Rhizocarpon geographicum*; light grey lines: *Caloplaca crenulatella*.

Fig. 5

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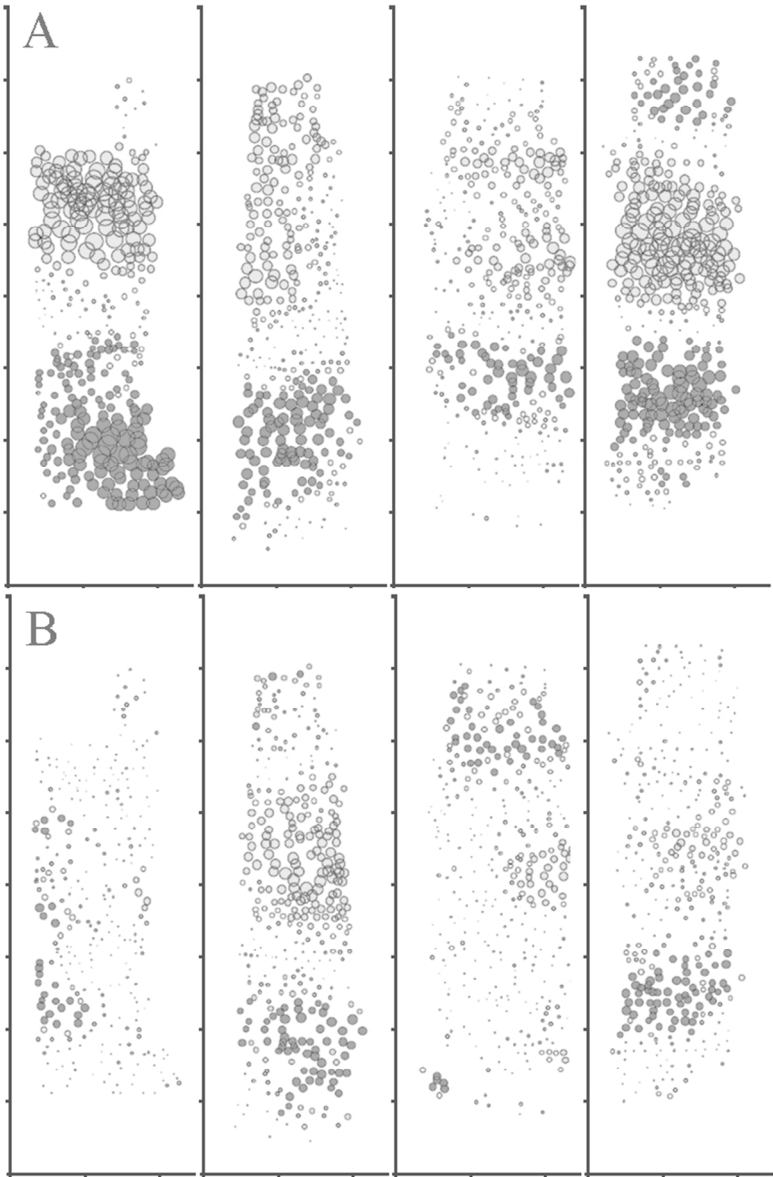


Fig. 6. Local *Gi bubble plots for *Rhizocarpon geographicum* (A) and *Caloplaca crenulatella* (B) on the stones along the four vertical transects on the wall of the medieval castle of Graines (axis ticks = 1 m). Bubble size is proportional to the intensity of autocorrelation at a given location. Dark grey bubbles indicate stones where the abundance of the species is higher than the mean values, while light grey bubbles indicate stones where the abundance of the species is lower than the mean values. White bubbles indicate stones where the abundance of the species is not significantly different from the mean values.

Fig. 6
69x99mm (300 x 300 DPI)

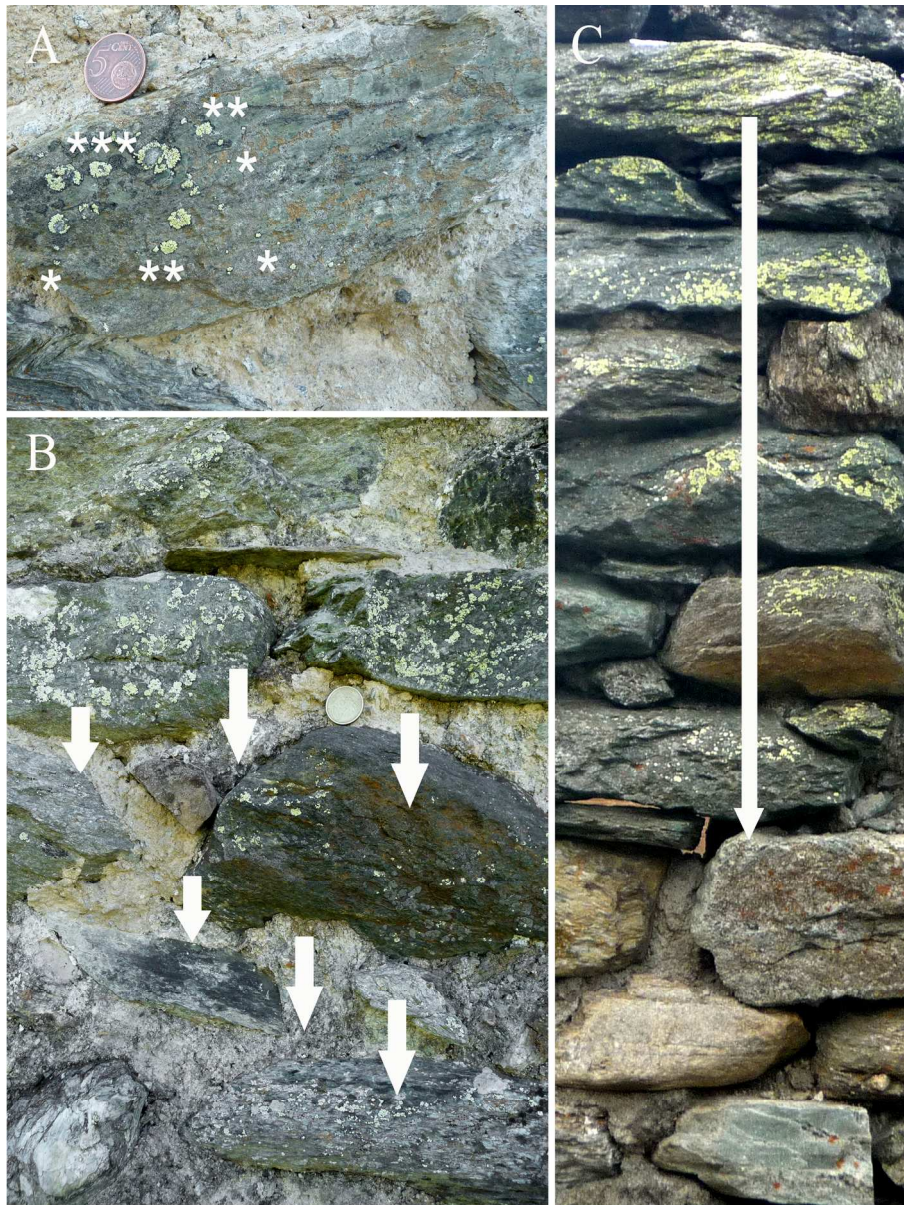


Fig. 7. Small-scale spatial distribution of *Rhizocarpon geographicum*. A, thalli with larger diameters (approx. 10 mm, ***) are surrounded by a progeny of thalli of lower dimensions (diameters approx. 6 mm, **, and <2 mm, *). B, C, rock blocks with high *Rhizocarpon* cover above blocks with decreasing cover and thallus dimensions at increasing distances (arrows).

Fig. 7

149x198mm (300 x 300 DPI)